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Modtaget

BACKGROUND OF THE INVENTION

Receptors are defined as proteinaceous macromolecules that perform a signal transducing function upon ligand binding. Many receptors are located on the outer cell membrane, others are located intracellularly. The substance which is bound by the receptor is called a ligand, a term which is definitionally meaningful only in terms of its counterpart receptor. The term "ligand" does not imply any particular molecular size or other structural or compositional feature other than that the substance in question is capable of binding, cleaving or otherwise interacting with the receptor in such a way that the receptor conveys information about the presence of the ligand to a target molecule. Stated alternatively, not all substances capable of binding a receptor are ligands, but all ligands are capable of binding a receptor. Receptors do not include such substances as immunoglobulins.

Receptors are believed to function by a process variously termed activation or signal transduction. A ligand binds to the ligand binding domain in such a way that the conformation of the receptor molecule changes. This conformational change, called activation, modifies the effect of the receptor on cytoplasmic components. Among changes brought about by receptor activation are changes in or development of receptor enzymatic activity.

Signalling proteins such as cAMP, IP3, kinases, and phosphatases are proteins ubiquitously found in all tissues. These proteins cascade by various pathways, the stimulus from ligand/receptor interaction down stream to cellular events, typically changing the enzymatic activity or functional state of effector molecules.

The pharmaceutical industry in recent years has oriented its research to focus on the role of receptors in disease or injury and to design drugs, generally low molecular weight substances, that are capable of binding to the receptors. Drugs identified in this initial screen are then tested for the activity *in vivo* or in tissue explants. As a result, conventional techniques do not lend themselves to large-scale screening. Tissue samples or isolated cells containing the target receptors, for example ovarian tissue, are costly to obtain, present in limited quantity, and difficult to maintain in a functionally viable state. Additionally, it is often difficult to reliably and reproducibly administer the candidate drug to tissue samples. Screening assays using primary explants in tissue culture are undertaken in larger scale than is possible with

tissue samples. However, it is more difficult to assay physiological effect and the assays are subject to interference from many sources, for example culture media or cultivation conditions. Finally, assays using receptors isolated from natural materials have the disadvantage that the receptor is subject to natural variability and suitable natural sources may not always
 5 be available. It is an object herein to provide readily reproducible, simple assay systems that can be practiced on a large scale for determining not only ligand binding but also the character of the binding as agonistic or antagonistic.

Similarly, meaningful clinical diagnosis often depends upon the assay of biologically active
 10 ligand without interference from inactive forms of the ligand, for example, ligands that have been subject to enzymatic or other processes in the test subject that change or even eliminate the activity of the ligand. Immunoassay methods are widely used in determining ligands in test samples. However, it is often quite difficult to identify antibodies that are able to discriminate between the active and inactive forms of a ligand. Receptors have frequently been
 15 used in place of antibodies as analyte binding reagents. However, not all substances that bind to receptors are necessarily capable of inducing receptor activity, i.e. active biologically. It is an object herein to provide a method that will identify ligands in clinical test samples which are active in inducing or inhibiting signal transduction by their receptors.

20 Cytoplasmic proteins can act as receptors or signalling molecules in cascading the stimulus from the ligand to cellular events. Various receptor or signalling protein types make use of different pathways (for example small G proteins, calcium fluxes, phosphatases and lipases) all of them resulting in changes of enzymatic activity or gene transcription.

25 Meiotic activating sterols (MAS) constitute active signalling molecules first identified in follicular fluid and in bull testicular tissue. The sterols are described by Byskov 1995 and Grøndahl et al. (Biol. Reprod. 58 (1998), 1297 *et seq.*) and in WO 96/00235, 96/27658, 97/00884, 98/28323, 98/54965 and 98/55498, more specifically in Claim 1 thereof, as being potent activators of the meiotic process. No receptors or signalling proteins have been described to di-
 30 rectly or indirectly signalling the meiotic effect of MAS sterols. Before this invention, the presence of the nature of a putative MAS receptor protein or a signalling protein has not been previously identified, although its presence has been suggested, for example, by Grøndahl et al. (Biol. Reprod. 62 (2000), 775 *et seq.*).

There remains considerable need for isolated and purified MAS receptor or MAS signalling protein, as well as systems capable of expressing MAS receptor or MAS signalling protein separate from other receptors. Further, it would be desirable to specifically identify the presence of MAS receptor or MAS signalling protein in cells and tissues, thereby avoiding the time-consuming, complex and non-specific functional pharmacological assays. It would also be desirable to screen and develop new agonists and/or antagonists specific for MAS receptor or MAS signalling protein for the use of antiinfertility or contraception drugs, but to date this has not been possible. Quite surprisingly, the present invention fulfils these and other related needs.

SUMMARY OF THE INVENTION

Now, the present invention provides the nucleotide sequence of the receptors or signalling proteins of meiotic acting sterols. The present invention provides isolated and substantially pure MAS receptors or MAS signalling proteins and fragments thereof. These receptors or signalling proteins have been shown to be involved in the gamete maturation process induced by FF-MAS, specifically inducing, upon ligand activation, germinal vesicle breakdown (GVB) in mouse oocyte cultured in vitro.

A MAS receptor is any protein related to the protein SAM1a or SAM1b that possess the same functional characteristic regarding the interaction with 3β -hydroxy-4,4-dimethylcholest-8,14,24-triene (hereinafter designated FF-MAS) or other endogenous meiosis activating sterols, for example, 3β -hydroxycholest-8,14-diene; 3β -hydroxy-4,4-dimethylcholest-8,24-diene; and 3β -hydroxycholest-8,24-diene, or their metabolites (as ligand). Functional characteristics include binding, receptor activation, and subsequent germinal vesicles breakdown (GVB) in oocytes. The amino acid sequence of SAM1a and SAM1b is stated in SEQ ID NO: 2 and SEQ ID NO: 4, below.

Having provided such receptors or signalling proteins in isolated or purified form, the invention also provides antibodies to the receptor or signalling protein, in the form of antisera and/or monoclonal antibodies.

In another aspect, the invention provides the ability to produce the MAS receptor or MAS signalling protein and polypeptides or fragments thereof by recombinant means. The expressed receptor or signalling protein or fragments may or may not have the biological activity of native receptor or signalling protein. Accordingly, isolated and purified polynucleotides are described which code for the receptor or signalling protein and fragment thereof, where the polynucleotides may be in the form of DNA, such as cDNA, or RNA. Based on these sequences, probes may be used to hybridise and identify these and related genes which encode MAS receptors or MAS signalling proteins. The probes may be full length cDNA or as small as form 14 to 25 nucleotide, more often though from about 40 to about 50 or more nucleotides.

In related embodiments, the invention concerns DNA constructs which comprise a transcriptional promoter, a DNA sequence which encodes the receptor or signalling protein or fragment, and a transcriptional terminator, each operably linked for expression of the receptor or signalling protein. For expression, the construct may also contain at least one signal sequence. Further, for large scale production, the expressed receptor or signalling protein may also be isolated from the cells by, for example, immunoaffinity purification.

Cells or bacteria which express the MAS receptor or MAS signalling proteins may also be used to identify compounds which can alter the receptor or signalling protein-mediated metabolism of a cell. Compounds may be screened for binding to the receptor or signalling protein, and/or for effecting a change in receptor or signalling protein-mediated metabolism in the host cell. Agonists and/or antagonists of the MAS receptor or MAS signalling proteins may also be screened in cell-free systems using purified receptor or signalling proteins or binding fragments thereof for the effect on ligand/receptor interaction or ligand/signalling protein interaction, or using reconstituted systems such micelles which also provide the ability to assess metabolic changes.

In yet other embodiments, the invention relates to methods for diagnosis, where the presence of a mammalian MAS receptor or MAS signalling protein in a biological sample may be determined. For example, a monospecific antibody which specifically binds the receptor or signalling protein is incubated with the sample under conditions conducive to immune com-

plex formation, which complexes are then detected, typically by means of a label such as an enzyme, fluorophore, radionuclide, chemiluminiscer, particle, or a second labelled antibody. Thus, means are provided for immunohistochemical staining of tissues, including ovarian or testicular tissues, for the subject receptor or signalling proteins.

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Based upon the similarity in sequence and the shared presence of a sterol binding domain at the protein level, the receptor proteins or signalling proteins of this invention can be said to belong to a novel super family of oxysterol binding proteins (hereinafter designated OSPB) recently published in *J.Lipid.Res.* 40 (1999), 2204. No function whatsoever in gamete maturation of either gender or regulation of any meiotic processes has been assigned to this OSPB family.

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BRIEF DESCRIPTION OF THE FIGURES

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SEQ ID NO: 1 and SEQ ID NO: 3 are the nucleotides of the cDNA from two MAS receptors or signalling peptides having the amino acid sequences stated in SEQ ID NO: 2 and SEQ ID NO: 4, respectively.

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DESCRIPTION OF THE PREFERRED EMBODIMENTS

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The present invention presents the means to identify agonists, and antagonists of the MAS receptor/ligand interaction or MAS signalling protein/ligand interaction by providing isolated MAS receptor or MAS signalling protein. The term "MAS receptor" refers to any proteins either derived from a naturally occurring MAS receptor, or which shares significant structural and functional characteristics peculiar to a naturally occurring MAS receptor. Such a receptor may result when regions of a naturally occurring receptor are deleted or replaced in such a manner as to yield a protein having a similar function. Homologous sequences, allelic variations, and natural mutants; induced point, deletion, and insertion mutants; alternatively expressed variants; proteins encoded by DNA which hybridise under high or low stringency conditions to nucleic acids which encode naturally occurring MAS receptor; proteins retrieved from naturally occurring materials; and closely related proteins retrieved by antisera

directed against MAS receptor proteins are also included. Similarly, this applies to MAS signalling proteins.

By MAS receptor "ligand" is meant a molecule capable of being bound by the ligand-binding domain of MAS receptor, a MAS receptor analogue, or chimeric MAS receptor as generally described in US Pat. No. 4,859,609, incorporated by reference herein. The molecule may be chemically synthesised or may occur in nature. Ligands may be grouped into agonists and antagonists. Agonists are those molecules whose binding to a receptor induces the response pathway within a cell. Antagonists are those molecules whose binding to a receptor blocks the response pathway within a cell.

By "isolated" MAS receptor or MAS signalling protein is meant to refer to MAS receptor or MAS signalling protein which is in other than its native environment such as a mammalian oocyte, including, for example, substantially pure MAS receptor as defined herein below. More generally, isolated is meant to include MAS receptor or MAS signalling protein as a heterologous component of a cell or other system. For example, MAS receptor or MAS signalling protein may be expressed by a cell transfected with a DNA construct which encodes MAS receptor or MAS signalling protein, separated from the cell and added to micelles which contain other selected receptor or signalling proteins.

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By purified MAS receptor or MAS signalling protein is meant MAS receptor or MAS signalling protein having a purity of at least 50%, preferably at least 80%, more preferred at least 90% (w/w).

In another aspect, the invention provides means for regulating the MAS receptor/ligand interaction or MAS signalling protein/ligand interaction, and thus treating, therapeutically and/or prophylactically, a disorder which can be linked directly or indirectly to MAS receptor or MAS signalling protein or to its ligands, such as FF-MAS. By virtue of having the receptor or signalling protein of the invention, agonists or antagonists may be identified which stimulate or inhibit the interaction of ligand with MAS receptor or with MAS signalling protein. With either agonists or antagonists, the metabolism and reactivity of cells which express the receptor or signalling protein are controlled, thereby providing a means to control meiosis in order to treat infertility or to achieve a novel principle of contraception.

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Thus, the invention provides screening procedures for identifying agonists or antagonists of events mediated by the ligand/MAS receptor or ligand/MAS signalling protein interaction.

Such screening assays may employ a wide variety of formats, depending to some extent on which aspect of the ligand, receptor or signalling protein interaction is targeted. For example, such assays may be designed to identify compounds which bind to the receptor or signalling protein and thereby block or inhibit interaction of the receptor or signalling protein and thereby block or inhibit interaction of the receptor or signalling protein with the ligand. Other assays can be designed to identify compounds which can substitute for ligand and therefore stimulate MAS receptor-mediated or MAS signalling protein-mediated intracellular pathways. Yet other assays can be used to identify compounds which inhibit or facilitate the association of MAS receptor or MAS signalling protein to FF-MAS and thereby mediate the cellular response to MAS receptor or MAS signalling protein ligand.

In one functional screening assay, the initiation of fertilisation activation events are monitored in eggs which have been injected with, for example, mRNA which codes for MAS receptor or MAS signalling protein and subsequently exposed to selected compounds which are being screened, in conjunction with or apart from an appropriate ligand. See generally, Kline *et al.*, *Science* 241 (1988), 464-467, incorporated herein by reference.

The screening procedure can be used to identify reagents such as antibodies which specifically bind to the receptor or signalling protein and substantially affect its interaction with ligand, for example. The antibodies may be monoclonal or polyclonal, in the form of antiserum or monospecific antibodies, such as purified antiserum or monoclonal antibodies or mixtures thereof. For administration to humans, for example, as a component of a composition for in vivo diagnosis or imaging, the antibodies are preferably substantially human to minimise immunogenicity and are in substantially pure form. By substantially human is meant generally containing at least about 70% human antibody sequence, preferably at least about 80% human, and most preferably at least about 90-95% or more of a human antibody sequence to minimise immunogenicity in humans.

Antibodies which bind MAS receptor or MAS signalling protein may be produced by a variety of means. The production of non-human antisera or monoclonal antibodies, for example,

murine, lagomorpha equine, etc. is well known and may be accomplished by, for example, immunising the animal with the receptor or signalling protein molecule or a preparation containing a desired portion of the receptor or signalling protein molecule, such as that domain or domains which contributes to ligand binding. For the production of monoclonal antibodies, antibody-producing cells obtained from immunised animals are immortalised and screened, or screened first for the production of antibody which binds to the receptor or signalling protein and then immortalised. As the generation of human monoclonal antibodies to human MAS receptor or MAS signalling protein antigen may be difficult with conventional techniques, it may be desirable to transfer antigen binding regions of the non-human antibodies, for example, the F(ab')₂ or hypervariable regions, to human constant regions (Fc) or framework regions by recombinant DNA techniques to produce substantially human molecules. Such methods are generally known in the art and are described in, for example, US Pat. No. 4,816,397, and EP publications 173,494 and 239,400, which are incorporated herein by reference. Alternatively, one may isolate DNA sequences which code for a human monoclonal antibody or portions thereof that specifically bind to the human receptor or signalling protein by screening a DNA library from human B cells according to the general protocol outlined by Huse et al., *Science* 246 (1989), 1275-1281, incorporated herein by reference, and then cloning and amplifying the sequences which encode the antibody (or binding fragment) of the desired specificity.

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In other embodiments, the invention provides screening assays conducted in vitro with cells which express the receptor or signalling protein. For example, the DNA which encodes the receptor or signalling protein or selected portions thereof may be transfected into an established cell line, for example, a mammalian cell line such as BHK and CHO, using procedures known in the art (see, for example, Sambrook et al, *Molecular Cloning, A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989, which is incorporated herein by reference). The receptor or signalling protein is then expressed by the cultured cells, and selected agents are screened for the desired effect on the cell, separately or in conjunction with an appropriate ligand such as FF-MAS or other MAS compounds.

Means for amplifying nucleic acid sequences which may be employed to amplify sequences encoding the receptor or signalling protein or portions thereof are described in US Pat. Nos. 4,683,195 and 4,683,202, incorporated herein by reference.

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In yet another aspect, the screening assays provided by the invention relate to transgenic mammals whose germ cells and somatic cells contain a nucleotide sequence encoding MAS receptor protein or signalling protein or a selected portion of the receptor which, for example, binds ligand. In yet a further aspect, the screening assays provided by the invention relate to
5 transgenic mammals where the nucleotide sequence encoding a MAS receptor or a MAS signalling protein is molecularly targeted to produce knock out animals with the phenotypical loss of the specific MAS signalling function. Preferentially, the molecular knock out is tissue specific to gonadal tissue (ovary or testes) and is timely controlled in the development, thus inducible. There are several means by which a sequence encoding, for example, the human
10 MAS receptor may be introduced into a non-human mammalian embryo or, alternatively, knocked out, some of which are described in, for example, US Pat. No. 4,736,866, Jaenisch, *Science* 240: 1468-1474 (1988) and Westphal et al., *Annu. Rev. Cell Biol.* 5: 181-196 (1989), which are incorporated herein by reference. The animal's cells then express the receptor or signalling protein and thus may be used as a convenient model for testing or screening se-
15 lected agonists or antagonists.

In another aspect the invention concerns diagnostic methods and compositions. By means of having the MAS receptor or MAS signalling protein molecule and antibodies thereto, a variety of diagnostic assays are provided. For example, with antibodies, including monoclonal
20 antibodies, to MAS receptor or MAS signalling protein, the presence and/or concentration of receptor or signalling protein in selected cells or tissues in an individual or culture of interest may be determined. These assays can be used in the diagnosis and/or treatment of diseases such as, for example, male infertility, female infertility, or by means of contraception in both gender.

25 Numerous types of immunoassays are available and are known to those skilled in the art, for example, competitive assays, sandwich assays, and the like, as generally described in, for example US Pat. Nos. 4,642,285; 4,376,110; 4,016,043; 3,879,262; 3,852,157; 3,850,752; 3,839,153; 3,791,932; and Harlow and Lane, *Antibodies, A Laboratory Manual*, Cold Spring
30 Harbor Publications, N.Y. (1988), each incorporated by reference herein. In one assay format, MAS receptor or MAS signalling protein is identified and/or quantified by using labelled antibodies, preferably monoclonal antibodies which are reacted with brain tissues, for example, ovarian or testicular tissue, oocyte preparations, or semen samples, and determining the

specific binding thereto, the assay typically being performed under conditions conducive to immune complex formation. Unlabeled primary antibody can be used in combination with labels that are reactive with primary antibody to detect the receptor or signalling protein. For example, the primary antibody may be detected indirectly by a labelled secondary antibody
5 made to specifically detect the primary antibody. Alternatively, the anti-MAS receptor-antibody or MAS signalling protein-antibody can be directly labelled. A wide variety of labels may be employed, such as radionuclides, particles (for example, gold, ferritin, magnetic particles, red blood cells), flourophores, chemiluminescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, and ligands (particularly haptens).

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The RNA encoding the MAS receptor DNA or MAS signalling protein DNA may be directly detected in cells with a labelled synthetic oligonucleotide probe targeting the MAS receptor (RNA) or MAS signalling protein RNA in a hybridisation procedure. Also, the polymerase chain reaction (Saiki *et al.*, *Science* 239 (1988), 487, and US Pat. No. 4,683,195, each refer-
15 ence is hereby incorporated by reference) may be used to amplify DNA sequences, which are subsequently detected by their characteristic size on agarose gels, Southern blot of these gels using MAS receptor DNA or MAS signalling protein DNA or a oligonucleotide probe, or a dot blot using similar probes. The probes may comprise from about 14 nucleotides to about 25 or more nucleotides, preferably, 40 to 60 nucleotides, and in some in-
20 stances a substantial portion or even the entire cDNA of MAS receptor or MAS signalling protein may be used. The probes are labelled with detectable signal, such as an enzyme, biotin, a radionuclide, fluorophore, chemiluminescer, and paramagnetic particle. High stringency in connection with hybridisation is obtained using the proper temperature and salt concentration.

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Kits can also be supplied for use with the receptor or signalling protein of the subject invention in the detection of the presence of the receptor or signalling protein or antibodies thereto, as might be desired in the case of autoimmune disease. Thus, antibodies to MAS receptor or MAS signalling protein, preferably monospecific antibodies such as monoclonal
30 antibodies, or compositions of the receptor or signalling protein may be provided, usually in lyophilised form in a container, either segregated or in conjunction with additional reagents, such as anti-antibodies, labels, gene probes, polymerase chain reaction primers and polymerase, and the like.

The following example is offered by way of illustration, not by limitation.

5 EXAMPLE 1

Microinjection of phosphorothionate oligonucleotides into mouse oocytes

Two antisense oligonucleotides (20 nucleotides) were utilized for microinjection: 5'-
 10 TCCACGATGGACGCCATCTT-3' and 5'-GCCAGCAGGAGAGCCATTCG-3', complementary to the kozak sequence of the mRNA encoded by the cDNA sequence herein designated *SAM1a* and *SAM1b*, respectively, both of which are defined in SEQ ID NO: 1 and SEQ ID NO: 3, respectively, shown below. In control experiments, the corresponding sense oligonucleotides were microinjected: 5'-AAGATGGCGTCCATCGTGGA-3' and 5'-
 15 CGAATGGCTCTCCTGCTGGC-3' for mRNA *SAM1a* and *SAM1b*, respectively. *SAM1a* antisense was co-injected with *SAM1b* antisense from a stock solution containing 1.25 µg/µl of each nucleotide in 10 % human serum albumin (hereinafter designated HSA) plus 5 mM Tris (pH value: 7.5). *SAM1a* sense was co-injected with *SAM1b* sense from a stock solution containing 1.25 µg/µl of each nucleotide in 10 % HSA plus 5 mM Tris (pH value: 7.5). Approximately 12 pg of each oligonucleotide (10 pl) were injected into the cytoplasm of individual
 20 germinal vesicle (GV)-stage oocytes loaded in a droplet of alpha-MEM supplemented with 0.8% HSA and 3 mM hypoxanthine under mineral oil in a 35 mm petri dish on the stage of an inverted microscope. The oocytes were obtained from the ovaries of 21-24 days old mice following 48 hours priming with follicle stimulating hormone (hereinafter designated FSH) as described by Grøndahl *et al.* 1998 in *Biol. Reprod.* 58 (1998), 1297 *et seq.* Oocytes were
 25 sucked on to a holding pipette (120 µm outer diameter and 20 µm inner diameter) and an injection pipette (Eppendorph, Hamburg, Germany) was fitted to a pressure microinjector (Eppendorph, Hamburg, Germany). The pipette holder was attached to a piezoelectric positioning system (Burleigh, NY, USA) mounted on a motorized micromanipulator (Luigs and
 30 Neumann, Ratingen, Germany). The injection pipette was pushed against the zona pellucida, and then a piezoelectric pulse was given, moving the injection pipette 20 µm forward. During this movement the pipette penetrated the zona pelludica and oolema and then a brief pressure pulse was applied to release a volume of approximately 10 µl into the oocytes cy-

toplasm. Injected oocytes were placed in a CO₂ incubator at 37 C for 20 hours before re-sumption of meiosis was triggered by addition of 10 µM FF-MAS to the hypoxanthine containing medium. The effect of FF-MAS was evaluated after 24 hours of further incubation as the number of oocytes in germinal vesicle breakdown (GVBD). The rationale for the 20 hours cultivation period following injection of antisense oligonucleotides is to allow for degradation of mRNA coding for SAM1a and SAM1b protein. Consequently, when the level of MAS receptor protein or MAS signalling protein is reduced in the oocytes, the MAS response is blunted (from 100% to 50%, *vide* the table below).

Table 1

Oligonucleotide	GVBD/GVBD+GV 10 µM FF-MAS (24 hours)
SAM1a + SAM1b Antisense	13/25
SAM1a + SAM1b Sense	10/10
Non-injected oocytes	26/29

As shown in Table 1, GVBD was inhibited by 50% in antisense injected oocytes compared to control (i.e., sense injected and non-injected oocytes). This result indicates a selective inhibition of the mRNAs coding for SAM1a and SAM1b by the antisense probe. Furthermore, these results indicate that SAM1a and SAM1b proteins are crucial involved in the MAS signalling, since a functional knock out of *de novo* protein synthesis of these molecules partly disrupt the MAS signals in oocytes.

SAM1a and SAM1b are two closely related proteins originating from the same gene which possesses complementary functions regarding MAS signalling in oocytes.

What is claimed is:

1. An isolated and/or purified polynucleotide molecule which hybridises at high stringency to an oligonucleotide of 25 or more contiguous nucleotides of SEQ ID NO: 1 or SEQ ID NO: 3 and which polynucleotide codes for a) a MAS receptor or MAS signalling protein; or b) a ligand binding domain of a MAS receptor or MAS signalling protein.
2. The polynucleotide of the preceding claim, which is a RNA antisense sequence.
3. The polynucleotide of claim 1, which is a cDNA sequence.
4. The polynucleotide according to any one of the preceding claims, which encodes a polypeptide displaying MAS receptor or MAS signalling protein activity.
5. The polynucleotide of claim 1, which encodes a MAS receptor or MAS signalling protein having the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4.
6. A probe which comprises an oligonucleotide of 25 or more contiguous nucleotides of SEQ ID NO: 1 or SEQ ID NO: 3 capable of specifically hybridising with a gene which encodes a MAS receptor or MAS signalling protein, or allelic and species variants thereof.
7. The probe of the preceding claim, which comprises from about 40 to about 60 nucleotides in length.
8. The probe according to any one of the two preceding claims, which is labelled to provide a detectable signal.
9. A probe of at least 12 nucleotides, said probe being capable of hybridising with nucleic acids which encode a MAS receptor or MAS signalling protein.
10. A DNA construct comprising a DNA sequence which hybridises at high stringency to

an oligonucleotide of 25 or more contiguous nucleotides of SEQ ID NO: 1 or SEQ ID NO: 3 and which encodes a) a MAS receptor or MAS signalling protein; or b) a ligand binding domain of a MAS receptor or MAS signalling protein.

- 5 11. The DNA construct of the preceding claim, wherein the DNA sequence encodes a MAS receptor or MAS signalling protein having the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4.
- 10 12. A cultured cell line, yeast or bacteria transformed or transfected with a DNA construct which comprises a DNA sequence which hybridises at high stringency to an oligonucleotide of 25 or more contiguous nucleotides of SEQ ID NO: 1 or SEQ ID NO: 3 and which encodes a) a MAS receptor or MAS signalling protein; or b) a ligand binding domain or a transmembrane domain of a MAS receptor or MAS signalling protein.
- 15 13. The cell line, yeast or bacteria according to the preceding claim, which does not express endogenous MAS receptor or MAS signalling proteins.
- 20 14. An MAS receptor or MAS signalling protein, a peptide fragment thereof or a salt thereof, which is isolated and/or purified.
- 25 15. The isolated and/or purified protein of the preceding claim, comprising the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4.
- 30 16. An isolated antibody which specifically binds to a MAS receptor or MAS signalling protein.
17. The isolated antibody of the preceding claim wherein said antibody is a monoclonal antibody.
18. The isolated antibody according to any one of the preceding two claims, which blocks the binding of MAS to a MAS receptor or MAS signalling protein.

19. A hybridoma which produces a monoclonal antibody according to any of the two preceding claims.
- 5 20. A method for detecting the presence of a compound or a salt thereof which has affinity for a MAS receptor or MAS signalling protein, comprising the steps of a) contacting the compound with the MAS receptor or MAS signalling protein, a peptide fragment thereof or a salt thereof; and b) measuring the affinity of said compound for the MAS receptor or MAS signalling protein.
- 10 21. The method of the preceding claim for detecting the presence of MAS antagonists, comprising the steps of a) exposing a compound in the presence of a MAS agonist including MAS to a MAS receptor or MAS signalling protein coupled to a response pathway under conditions and for a time sufficient to allow binding of the compound to the receptor or MAS signalling protein and an associated response through the
15 pathway; and b) detecting a reduction in the stimulation of the response pathway resulting from the binding of the compound to the MAS receptor or MAS signalling protein, relative to the stimulation of the response pathway by the MAS agonist alone and therefrom determining the presence of a MAS antagonist.
- 20 22. The method of any one of the two preceding claims for detecting the presence of MAS agonists, comprising the steps of a) exposing a compound in the presence of a MAS antagonist to a MAS receptor or MAS signalling protein coupled to a response pathway under conditions and for a time sufficient to allow binding of the compound to the receptor or MAS signalling protein and an associated response through the
25 pathway; and b) detecting an increase of the stimulation of the response pathway resulting from the binding of the compound to the MAS receptor or MAS signalling protein, relative to the stimulation of the response pathway by the MAS antagonist alone and therefrom determining the presence of a MAS agonist.
- 30 23. A compound or a salt thereof which has affinity for the MAS receptor or a MAS signalling peptide and which compound or salt is detected by the method according to claim 20 or 21.

24. A method for producing a MAS receptor or MAS signalling protein having the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4, which comprises a) growing cells, yeast or bacteria transformed or transfected with a DNA construct which comprises a DNA sequence of SEQ ID NO: 1 or SEQ ID NO: 3 coding for the expression of the MAS receptor or MAS signalling protein, and b) isolating the receptor or MAS signalling protein from the cells.
25. The method according to the preceding claim, wherein the MAS receptor or MAS signalling protein is isolated by immunoaffinity purification.
26. A kit for screening a compound or a salt thereof which has affinity for a MAS receptor or MAS signalling protein, which contains the MAS receptor or MAS signalling protein, the peptide fragment thereof or a salt thereof.

SEQ ID NO: 1

SAM1a DNA

5 TTTGTTGTCATTGGCGGCTCCCAAGATGGCGTCCATCGTGGAAGGGCCGCTGAGCAAA
TGGACTAACGTGATGAAGGGATGGCAGTATCGTTGGTTCGTGCTGGACTACAATGCAG
GGCTGCTCTCCTACTACACGTCCAAGGACAAAATGATGAGAGGCTCTCGAAGAGGATG
CGTTAGACTCAGAGGAGCTGTGATTGGTATAGACGACGAGGACGACAGCACCTTCACA
ATCACTGTGATCAGAAAACCTTCCACTTCCAGGCTCGAGATGCAGACGAGCGAGAGA
10 AGTGGATCCATGCCTTAGAAGAACTATTCTTCGCCATACTCTTCAGCTTCAAGGTTTGG
ATTGAGGATTCATCCCCAGTGTCCAAGACTTTGATAAGAACTTACCGAGGCTGACGCG
TACCTGCAGATCTTGATAGAACAATTAAGCTTTTTGATGACAAGCTTCAAAATTGTAAA
GATGATGAACAGAGAAAGAAAGTTGAACTCTCAAAGACACAACAAATAGCATGGTAGA
ATCAATTAACACTGCATTGTGTTGCTACAGATTGCTAAAAGTACTATTAATCCTGTAGAT
15 GCAATATACCAGCCTAGTCCCTTGGAACTGTGATCAGCACAATGCCTTCCCAGACTGC
CTTACCTCCAGAACCCGCTCAGTTGTGTAAGTCAGAGCAGCGTCCATCTTCCTTACCTG
TTGGACCTGTGTTAGCTACCTTGGGACATCATCAGACTCCAACACCAAATAGTACAGGC
AGTGGGAACCTACCCACCTAGCAGCAGTCTGACTCCTCCCAGCCATGTCAACTTGTCTCC
AAATACAGTCCCAGAGTTCTCTTACTCTAGCAGTGAAGATGAGTTCTATGATGCTGATGA
20 ATTCCATCAAAGTGGCTCGTCCCCAAAGCGCTTAATAGATTCTTCTGGATCTGCCTCAGT
CTTGACACACAGCAGCTCCGGAAATAGCTTAAACGCCCAGATACCACAGAGTCTCTGA
ATTCCTCCATGTCCAATGGCACAAGCGATGCTGATCTTTTTGACTCACATGACGACAGA
GATGATGATGGGGAGGCTGGGTGAGTGGAGGAGCACAAGAGCGTTATCATGCACCTCT
TATCACAAGTCAGGCTGGGGATGGACCTCACAAAGGTAGTTCTTCCAACGTTTATTCTC
25 GAGAGAAGATCTCTGTTAGAAATGTATGCAGACTTTTTCGCACATCCAGACCTGTTTCGT
GAGCATTAGTGATCAGAAGGATCCCAGGGATCGAATGGTTCAGGTTGTGAAATGGTAC
CTCTCGGCCTTCCATGCAGGAAGGAGAGGATCGGTGGCCAAAAAGCCGTACAATCCTA
TTTTGGGTGAGATCTTTCAGTGTCACTGGACGTTGCCGAATGATACTGAAGAGAACGCA
GAGCTCGTTTTCAGAAGGGCCGGTTCCCTGGGTTTTCTAAGAACAGTGTAACATTTGTGGC
30 TGAGCAAGTTTTCCACCATCCGCCATTTTCCAGCCTTTTATGCTGAGTGTTTTAACAAGAA
GATACAATTCAATGCTCATATCTGGAATAAATCAAATTCCTTGGGATGTCAATTGGGGT
ACACAACATAGGTCAGGGCTGTGTCTCGTGTCTGGAGTACGATGAGCACTACATCCTCA
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Novo Nordisk A/S

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ABSTRACT

Two receptors of meiosis activating sterols designated SAM1a and SAM1b have been identified.

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 Thr Thr Glu Ser Leu Asn Ser Ser Met Ser Asn Gly Thr Ser Asp Ala
 225 230 235 240
 Asp Leu Phe Asp Ser His Asp Asp Arg Asp Asp Asp Gly Glu Ala Gly
 245 250 255

Ser Val Glu Glu His Lys Ser Val Ile Met His Leu Leu Ser Gln Val
 260 265 270
 5 Arg Leu Gly Met Asp Leu Thr Lys Val Val Leu Pro Thr Phe Ile Leu
 275 280 285
 Glu Arg Arg Ser Leu Leu Glu Met Tyr Ala Asp Phe Phe Ala His Pro
 290 295 300
 10 Asp Leu Phe Val Ser Ile Ser Asp Gln Lys Asp Pro Arg Asp Arg Met
 305 310 315 320
 Val Gln Val Val Lys Trp Tyr Leu Ser Ala Phe His Ala Gly Arg Arg
 325 330 335
 15 Gly Ser Val Ala Lys Lys Pro Tyr Asn Pro Ile Leu Gly Glu Ile Phe
 340 345 350
 Gln Cys His Trp Thr Leu Pro Asn Asp Thr Glu Glu Asn Ala Glu Leu
 355 360 365
 Val Ser Glu Gly Pro Val Pro Trp Val Ser Lys Asn Ser Val Thr Phe
 370 375 380
 25 Val Ala Glu Gln Val Ser His His Pro Pro Ile Ser Ala Phe Tyr Ala
 385 390 395 400
 Glu Cys Phe Asn Lys Lys Ile Gln Phe Asn Ala His Ile Trp Thr Lys
 405 410 415
 30 Ser Lys Phe Leu Gly Met Ser Ile Gly Val His Asn Ile Gly Gln Gly
 420 425 430
 Cys Val Ser Cys Leu Glu Tyr Asp Glu His Tyr Ile Leu Thr Phe Pro
 435 440 445
 Asn Gly Tyr Gly Arg Ser Ile Leu Thr Val Pro Trp Val Glu Leu Gly
 450 455 460
 40 Gly Glu Cys Asn Ile Asn Cys Ser Lys Thr Gly Tyr Ser Ala Asn Ile
 465 470 475 480
 Val Phe His Thr Lys Pro Phe Tyr Gly Gly Lys Lys His Arg Ile Thr
 485 490 495
 45 Ala Glu Ile Phe Ser Pro Asn Asp Lys Lys Ser Phe Cys Ser Ile Glu
 500 505 510
 Gly Glu Trp Asn Gly Ile Met Tyr Ala Lys Tyr Ala Thr Gly Glu Asn
 515 520 525
 50 Thr Val Phe Val Asp Thr Lys Lys Leu Pro Ile Ile Lys Lys Lys Val
 530 535 540

Arg Lys Leu Glu Asp Gln Asn Glu Tyr Glu Ser Arg Ser Leu Trp Lys
 545 550 555 560
 5 Asp Val Thr Phe Asn Leu Lys Ile Arg Asp Ile Asp Ala Ala Thr Glu
 565 570 575
 Ala Lys His Arg Leu Glu Glu Arg Gln Arg Ala Glu Ala Arg Glu Arg
 580 585 590
 10 Lys Glu Lys Glu Ile Gln Trp Glu Thr Arg Leu Phe His Glu Asp Gly
 595 600 605
 Glu Cys Trp Val Tyr Asp Glu Pro Leu Leu Lys Arg Leu Gly Ala Val
 15 610 615 620
 Lys His
 625

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Novo Nordisk A/S

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